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TABLE OF CONTENTS

EXECUTIVE SUMMARY 3
1 INTRODUCTION 5
2 REVIEW METHODS AND MATERIALS 6
3 REVIEW RESULTS 7
3.1 Sznajder et al. (2022) 7
3.1.1 Study Overview 7
3.1.2 Study Objectives/Specific Aims/Scope 7
3.1.3 Study Methods 7
3.1.3.1 Design & Setting 7
3.1.3.2 Exposure 8
3.1.3.3 Outcomes 9
3.1.3.4 Covariates 9
3.1.3.5 Sample Size/Power 9
3.1.3.6 Statistical Analysis 10
3.1.4 Study Results 10
3.1.4.1 Sample Characteristics 10
3.1.4.2 Factors Associated with Exposure or Outcomes 10
3.1.4.3 APAP and Behavioral Problems 11
3.1.5 Study Conclusions 11
3.2 Theunissen et al. (2022) 11
3.2.1 Methods and Results 11
3.3 Zafeiri et al. (2022) 12
3.3.1 Methods and Results 12
4 REVIEW DISCUSSION 14
4.1 Sznajder et al. (2022) 14
4.2 Theunissen et al. (2022) 16
4.3 Zafeiri et al. (2022) 16
4.4 Overall Discussion 17
5 CONCLUSIONS 17
6 APPENDICES 19
6.1 Appendix A. Sznajder et al. Study Summary Table 19

6.2 Appendix B. Maternal and neonatal characteristics overall and by acetaminophen use during pregnancy (N = 2,422). 21

6.3 Appendix C. Association of potentially confounding variables with each of the CBCL syndrome scale outcomes. 22

6.4 Appendix D. Association between acetaminophen exposure during pregnancy and neurobehavioral problems in 3-year-old children. 23

6.5 Appendix E. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Emotionally Reactive" 23

6.6 Appendix F. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Anxious/Depressed" 24

6.7 Appendix G. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Somatic Complaints" 24

6.8 Appendix H. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Withdrawn"..... 25

6.9 Appendix I. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Sleep Problems" 25

6.10 Appendix J. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Attention Problems" 26

6.11 Appendix K. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Aggressive Behavior" 26

6.12 Appendix L. Study Criteria Indicative of Higher Quality Studies..... 27

6.13 Appendix M. References..... 28

EXECUTIVE SUMMARY

On September 26, 2022, the Office of Media Affairs within the Office of External Affairs received a media request about the safety of acetaminophen (APAP) use during pregnancy. The request was prompted by an article published by Sznajder et al. (2022) in PLoS One. On September 27, 2022, the Division of Nonprescription Drugs (DNPD) requested that the Office of Surveillance and Epidemiology (OSE) review the article published in PLoS One. Between 2014 and 2022, the Division of Epidemiology I (DEPI-I) conducted five literature reviews on in utero APAP exposure and neurobehavioral and urogenital outcomes, with the most recent review covering articles published from January 1, 2016, through December 1, 2021. The purpose of this review is for DEPI-I to evaluate the PLoS One article along with articles published since the most recent DEPI-I review (i.e., from December 1, 2021, through November 18, 2022), and to determine if any study findings affect conclusions from prior DEPI-I reviews about the safety of APAP use during pregnancy with respect to neurobehavioral and urogenital outcomes.

The study by Sznajder et al. (2022) is a prospective cohort study that examined the association between APAP use during pregnancy and behavioral problems at 36 months of age. APAP use since the start of pregnancy was assessed at one point in time, in the third trimester, and behavioral problems were assessed with the Child Behavior Checklist (CBCL) at 36 months of age. Out of a sample of 2,422 women in Pennsylvania, 1,011 (41.7%) reported APAP use during pregnancy. After adjustment for confounders, including stress during pregnancy, any APAP use was significantly associated with sleep problems and attention problems but not emotionally reactive, anxious/depressed, somatic complaints, withdrawn, or aggressive behavior.

Two additional studies were included in this review. The first by Theunissen et al. (2022) examined prenatal risk factors, including APAP use, for depressive symptoms in eight-year old children in New Zealand. APAP use during pregnancy was retrospectively assessed through interviews typically conducted in the third trimester and depressive symptoms were assessed with a screening tool (Centre for Epidemiological Studies Depression Scale for Children [CESD-10]). In the final, predictive model, a significant association was found between APAP use and CESD-10 scores (n=3,925). Zafeiri et al. (2022) explored the relationship between over-the-counter analgesics and adverse neonatal outcomes, including cryptorchidism and hypospadias. The retrospective cohort study, conducted in the United Kingdom, used medical notes to capture APAP exposure, which was assessed at the first antenatal visit, as well as study outcomes for 151,141 pregnancies. Among males, after adjustment for confounders, there was no association between APAP use and cryptorchidism or hypospadias.

Findings in the literature on the associations between APAP use during pregnancy and neurobehavioral and urogenital outcomes remain mixed. The study by Sznajder et al. (2022) is the first to document an association between APAP use and sleep problems and suggests that stress during pregnancy may be an important confounder. Theunissen et al. (2022) is the first to examine depressive symptoms as an outcome and found an association between APAP and depressive symptoms at eight years of age. The clinical significance of these associations is unclear. The studies are also limited by their one-time assessments of APAP exposure and their lack of adjustment for key confounders, namely indications like fever and headache/migraine. Overall, the three new studies reviewed are limited and do not change DEPI-I's conclusions from

its most recent review— the limitations and inconsistent findings of current observational studies of APAP and neurobehavioral and urogenital outcomes are unable to support a determination of causality.

1 INTRODUCTION

On September 26, 2022, the Office of Media Affairs (OMA), within the Office of External Affairs (OEA), received a media request about the safety of acetaminophen (APAP) use during pregnancy. The media request was prompted by the following article, which was embargoed at the time of the request:

- Sznajder KK, Teti DM, Kjerulff KH. Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. PLoS One. 2022 Sep 28;17(9):e0272593.

On September 27, 2022, the Division of Nonprescription Drugs (DNPD) requested that the Office of Surveillance and Epidemiology (OSE) review the article published in PLoS One.

Between 2014 and 2022, the Division of Epidemiology I (DEPI-I) conducted several literature reviews on in utero APAP exposure and neurobehavioral and urogenital outcomes:

- Taylor LG, Wang C. Review of study of acetaminophen use in pregnancy and risks of ADHD in offspring. May 15, 2014. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 3507534.

- Mosholder AD, Taylor LG, Pinheiro SP. Acetaminophen use in pregnancy and ADHD in offspring. March 18, 2015. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 3718011.

- Mosholder AD, Taylor LG, Wang C. Neurodevelopmental outcomes following prenatal acetaminophen exposure. October 14, 2016. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 3999031.

- Mosholder AD, Leishear K, Sandhu SK. Urogenital outcomes with in utero acetaminophen exposure. January 7, 2019. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 4372262.

- Abraham D, Mosholder AD, Leishear White K, Sandhu SK. Functional neurobehavioral outcomes and urogenital outcomes associated with prenatal acetaminophen exposure. July 15, 2022. Silver Spring (MD), U.S. Food and Drug Administration. Available in Lifecycle Signal Tracker (LiST) under NISS ID: 1001355.[1]

APAP is an analgesic and antipyretic available alone or in combination products in both over-the-counter and prescription formulations.[2] A summary of past DEPI-I reviews, APAP use in

[1] Covered articles published from January 1, 2016, through December 1, 2021.

[2] Drugs@FDA: FDA-Approved Drugs. Silver Spring (MD), U.S. Food and Drug Administration. Accessed November 3, 2022 at: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm; DailyMed. Bethesda (MD), National Library of Medicine. Accessed December 9, 2022 at: https://dailymed.nlm.nih.gov/dailymed/

pregnancy, and the regulatory history of APAP can be found in the July 15, 2022, review conducted by DEPI-I.[3] The most recent review concluded that the limitations and inconsistent findings of current observational studies of APAP and neurobehavioral and urogenital outcomes are unable to support a determination of causality. The review recommended that nonclinical toxicological studies be conducted to better understand the impact of prenatal APAP exposure on neurobehavioral and urogenital development.

The purpose of this review is for DEPI-I to evaluate the PLoS One article along with articles published since the most recent DEPI-I review, and to determine if the any study findings affect conclusions from prior DEPI-I reviews about the safety of APAP use during pregnancy with respect to neurobehavioral and urogenital outcomes.

2 REVIEW METHODS AND MATERIALS

This review summarizes and discusses the following study published by Sznajder et al. (2022) in PLoS One:

- Sznajder KK, Teti DM, Kjerulff KH. Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. PLoS One. 2022 Sep 28;17(9):e0272593.

To update DEPI's most recent review, a PubMed literature search was conducted restricting to human studies, published in English from December 1, 2021, through November 18, 2022. The following search terms were used:

- ((acetaminophen OR paracetamol)) AND (pregnancy)
- ((acetaminophen OR paracetamol)) AND (birth outcomes)
- ((acetaminophen OR paracetamol)) AND (neurodevelopment)
- ((acetaminophen OR paracetamol)) AND (urogenital)
- ((acetaminophen OR paracetamol)) AND (prenatal)

The search identified 96 articles. The title and/or abstract was reviewed for each article for review inclusion. References from relevant, review articles identified in the literature search (n=2) (1, 2) were also screened for inclusion. No additional, relevant articles were identified that were published in 2016 or later that had not been previously reviewed by DEPI-I. Articles were excluded from the review primarily due to not being original research articles or due to a lack of relevancy. For example, studies excluded focused on APAP use in infants (not during pregnancy), pregnancy outcomes, caesarian section/labor and delivery pain management, utilization patterns, and patent ductus arteriosus. Despite search restrictions, a few excluded

[3] Abraham D, Mosholder AD, Leishear White K, Sandhu SK. Functional neurobehavioral outcomes and urogenital outcomes associated with prenatal acetaminophen exposure. July 15, 2022. Silver Spring (MD), U.S. Food and Drug Administration. Available in Lifecycle Signal Tracker (LiST) under NISS ID: 1001355.

studies were animal studies or cellular studies. One additional article (3), which was found to be a mechanistic study, was excluded upon review of the full article.

Two relevant epidemiologic studies, published between December 1, 2021, and November 18, 2022, were identified for review:

- Zafeiri A, Raja EA, Mitchell RT, et al. Maternal over-the-counter analgesics use during pregnancy and adverse perinatal outcomes: Cohort study of 151 141 singleton pregnancies. BMJ Open. 2022 May 3;12(5):e048092.

- Theunissen G, D'Souza S, Peterson ER, et al. Prenatal determinants of depressive symptoms in childhood: Evidence from Growing Up in New Zealand. J Affect Disord. 2022 Apr 1;302:41-49.

3 REVIEW RESULTS

3.1 SZNAJDER ET AL. (2022)

3.1.1 Study Overview

This prospective cohort study, supported by grant funding through the National Institutes of Health, examined the association between APAP use during pregnancy and behavioral problems at 36 months of age. APAP use since the start of pregnancy was assessed at one point in time, in the third trimester, and behavioral problems were assessed with the Child Behavior Checklist (CBCL) at 36 months of age. A study summary is provided in Appendix A.

3.1.2 Study Objectives/Specific Aims/Scope

The study objective was, "[T]o examine associations between prenatal acetaminophen exposure and offspring neurobehavioral problems at the age of 3 years, with focus on the potentially confounding effects of prenatal stress" (4).

3.1.3 Study Methods

3.1.3.1 Design & Setting

3.1.3.1.1 Study Type

Prospective, cohort study.

3.1.3.1.2 Population & Time Period

The study sample is derived from the First Baby Study (FBS). FBS is a longitudinal cohort study of nulliparous pregnant women that aims to assess the impact of delivery type on later reproduction. FBS recruited[4] and enrolled 3,006 women who delivered in 2009, 2010, or 2011 at 78 different hospitals in Pennsylvania. FBS used the following inclusion and exclusion criteria:

Inclusion Criteria:

- Women 18-35 years of age
- Speak English or Spanish
- Plan to deliver at hospital in Pennsylvania

Exclusion Criteria:

- <18 years of age
- Prior pregnancy (≥20 weeks gestation)
- Planned home delivery or delivery at non-hospital affiliated birthing center
- Plan for child to be adopted
- Delivery <34 weeks gestation

For this study of prenatal APAP exposure, participants were further restricted to those who completed a 36-month postpartum questionnaire and had some CBCL outcome data available. If a single CBCL syndrome scale item was skipped, scores were generated with individual mean imputation. If more than one item was skipped, then that woman was excluded from the analysis for that respective scale.

3.1.3.1.3 Protected Health Information (PHI) Requirement

The study received institutional review board approval from the Penn State College of Medicine and entities involved in recruitment. Women provided signed, informed consent.

3.1.3.2 Exposure

Medication exposure since the start of pregnancy was assessed through telephone interviews conducted by trained professional interviewers at one point in time in the third trimester (mean time of interview=35.2 weeks gestation, standard deviation [SD]=1.6 weeks). Women were asked, "Have you taken prescription or non-prescription medications other than vitamins at least occasionally since you became pregnant?" (4). Women could report the medication, dose,

[4] Recruitment through childbirth classes, newspaper advertisements, mailings, hospital notifications, and posters (in clinical and public settings).

frequency, and reason for taking up to ten medications. There was insufficient detail available to categorize dose and frequency of exposure for medications. Exposed women were those who reported taking one or more medications (prescription or non-prescription) that contain APAP.

3.1.3.3 Outcomes

The CBCL instrument was completed through parental interviews at 36-months postpartum. Items were grouped and scored as the following syndrome scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behavior. Due to skewed and kurtotic distributions, scores were dichotomized using the 80th percentile as a cutoff score.

3.1.3.4 Covariates

Covariate data, which were verified, when possible, were obtained from interviews, birth certificates, and hospital discharge data. Covariates included:

- Modified Psychosocial Hassles Scale (measure of stress during pregnancy)
    - Stress was categorized, based on total scores, as high stress, medium stress, and low stress
- Edinburgh Depression Scale (measure of depression during pregnancy)
- Maternal socio-demographic factors (e.g., age, education level, race/ethnicity, marital status, insurance type)
- Pre-pregnancy health history (e.g., body mass index, diagnosis of anxiety or depression)
- Health habits during pregnancy (e.g., smoking and alcohol consumption)
- Co-medications – one or more prescription and/or non-prescription medication (excludes vitamins and APAP)
- Reason took medication in pregnancy (categories include fever, infection, muscle pain, headache/migraine, cold/allergies, trouble sleeping thyroid conditions, anemia, asthma, nausea)
    - This information was collected for each medication reported; however, reasons for taking <u>any</u> medication were considered, instead of the specific indication for the exposure of interest (e.g., APAP).
- Labor induction and delivery type
- Pregnancy and delivery complications
- Newborn characteristics (e.g., gestational age, birthweight, 5-minute Apgar score, assisted ventilation, jaundice, neonatal intensive care unit [NICU] admission, sex)

3.1.3.5 Sample Size/Power

None provided.

3.1.3.6 Statistical Analysis

Analysis was performed in SAS v9.4 and SPSS. Two-sided Chi-square tests were used to compare maternal and newborn characteristics by APAP exposure. Multivariable logistic regression was used to examine associations between APAP use during pregnancy and CBCL syndrome scale outcomes. Confounders in the regression models were covariates with statistically significant associations with APAP exposure and the respective CBCL syndrome scale outcome. Fever and headache/migraine were collinear with APAP use during pregnancy and were omitted from regression models.

3.1.4 Study Results

3.1.4.1 Sample Characteristics

Of the 3,006 women in FBS, 2,423 (80.6%) completed the 36-month interview and 2,422 had some outcome data available. The number of women excluded from a respective syndrome scale analysis due to missing data items ranged from 3 (sleep problems and attention problems, withdrawn) to 36 (aggressive behavior).

Overall, 1,011 (41.7%) women in the study sample used APAP during pregnancy. Women most commonly reported using medications for headache/migraine. The majority of women were between 25 and 29 years of age (43.0%), had a college degree or higher (63.2%), were married (77.9%), were White non-Hispanic (88.1%), and had private insurance (83.7%). Additional characteristics are presented in Appendix B.

Compared to pregnant nulliparous women in all of Pennsylvania, the women in FBS were more educated, more likely to be White non-Hispanic, more likely to be married, and more likely to have private insurance. Women who remained in FBS were older, more educated, more likely to be White, more likely to be married, and more likely to have private insurance.

3.1.4.2 Factors Associated with Exposure or Outcomes

Women with APAP use during pregnancy were significantly more likely to report taking medications in pregnancy for fever, infection, muscle pain, headache/migraine, cold/allergies, trouble sleeping, and thyroid conditions, than those who did not report APAP use. Maternal age and pre-pregnancy BMI differed by APAP use. APAP users were significantly more likely to be White non-Hispanic, have private insurance, consume alcohol during pregnancy, take non-prescription drugs (not including vitamins or APAP), have a pre-pregnancy diagnosis of anxiety or depression, report more stress during pregnancy, to have labor induced, and to have a cesarean section. Additional data are presented in Appendix B.

Associations between maternal/newborn characteristics and CBCL syndrome scale outcomes are presented in Appendix C. The most common outcome was attention problems (30.0%) and the

least common was somatic complaints (16.5%). The only characteristics significantly[5] associated with all syndrome scale outcomes were alcohol consumption during pregnancy, pre-pregnancy anxiety or depression, and stress during pregnancy.

3.1.4.3 APAP and Behavioral Problems

There were no significant associations in unadjusted or adjusted models between APAP use during pregnancy and emotionally reactive, anxious/depressed, somatic complaints, or aggressive behavior. APAP use was only significantly associated with withdrawn behavior in unadjusted models. APAP use was significantly associated, in both unadjusted and adjusted models, with sleep problems (unadjusted odds ratio [OR]=1.26, 95% confidence interval [CI]: 1.04, 1.54; adjusted odds ratio [aOR]=1.23, 95% CI: 1.01, 1.51) and attention problems (OR=1.27, 95% CI: 1.06, 1.51; aOR=1.21, 95% CI: 1.01, 1.45). Additional model results are presented in Appendix D through K. Given stress (as a categorical variable) was associated with APAP exposure and all outcomes in bivariate analysis, categorical stress was included in all adjusted models. In all adjusted models, the risk of behavioral problems increased with increasing stress level; high psychosocial stress during pregnancy was significantly associated with all CBCL syndrome scale outcomes, compared to low stress during pregnancy.

3.1.5 Study Conclusions

The authors conclude that the study found an association between APAP use during pregnancy and child behavioral problems at the age of 3 years, specifically attention problems and sleep problems. The authors recommended that providers should consider the risks and benefits of APAP use during pregnancy.

3.2 THEUNISSEN ET AL. (2022)

3.2.1 Methods and Results

The objective of the study by Theunissen et al. (2020) was, [T]o identify prenatal risk factors of depressive symptoms at age 8 in a diverse and contemporary cohort, with particular emphasis on maternal mental health and maternal lifestyle factors" (5). This longitudinal cohort study was conducted using the Growing Up in New Zealand (GUiNZ) cohort. The GUiNZ cohort includes births from 6,822 pregnant women from three New Zealand health regions with estimated dates of delivery between April 25, 2009, and March 25, 2020. The study received ethics committee approval and mothers provided written, informed consent. Data were collected through computer-assisted, in-person interviews or telephone interviews. During the antenatal data collection wave (interview typically conducted in third trimester (6)), information was collected

[5] The authors did not provide a p-value threshold for bivariate analysis and p-values were not provided in table in Appendix C (statistically significant associations were bolded)

on maternal antenatal depression, perceived stress, pre-pregnancy BMI, multivitamin use during pregnancy, folate use pre-pregnancy and during pregnancy, medication use during pregnancy (e.g., APAP, antidepressants, aspirin [dichotomized as yes/no]), smoking, second-hand smoke exposure, alcohol consumption, ethnicity, age, education, neighborhood deprivation index, and parity, along with whether the pregnancy was planned or unplanned. During the eight-year data collection wave, children were screened for depressive symptoms with the 10-item short-form of the Centre for Epidemiological Studies Depression Scale for Children (CESD-10)[6]. The association between each predictor and the outcome was assessed with univariate analysis, and significant predictors ($p<0.05$) in the univariate analysis were included in the final, hierarchical linear regression models. Final models were constructed adding a block of sociodemographic factors, followed by a block of maternal mental health factors, and lastly a block of maternal lifestyle factors (including APAP use).

A total of 5,010 children contributed to the eight-year data collection wave; the final study sample was an analysis of 3,925 children with complete predictor and outcome information.[7] APAP use during pregnancy was reported by 67.2% of the sample. In univariate analysis, children from pregnancies with APAP exposure had statistically significantly higher CESD-10 scores (mean=6.56, standard deviation [SD]=4.44) than those with no exposure (mean=6.08, SD=4.35) ($p<0.001$). The final model included APAP use, antenatal depression[8], perceived stress, folate use, vitamin use, alcohol consumption, smoking, second-hand smoke, and pre-pregnancy BMI, and controlled for rurality, deprivation, parity, child sex, mother's ethnicity, mother's age, planned pregnancy status, and mother's education. In the final model, there was a significant association between APAP use and higher CESD-10 scores (B=0.33, standard error [SE]=0.15, p=0.03). Other significant predictors included perceived stress (B=0.039, SE=0.13, $p<0.01$), continued smoking during pregnancy (B=0.93, SE=0.31, $p<0.01$), and overweight/obese pre-pregnancy BMI (B=0.42, SE=0.15, $p<0.01$).

3.3 ZAFEIRI ET AL. (2022)

3.3.1 Methods and Results

In Zafeiri et al. (2022), the retrospective cohort study aimed, "To identify any association between in utero exposure to five over-the-counter (non-prescription) analgesics (paracetamol,

[6] Authors conducted a confirmatory factor analysis to validate CESD-10 in this study sample

[7] Children not in the study sample due to attrition were more likely to be second (or subsequent) children and be from unplanned pregnancies; they were also more likely to have mothers who had depressive symptoms, were non-European, were younger, resided in higher deprivation areas, had less education, were more likely to smoke during pregnancy. It is unclear if this comparison is made between the 3,925 children in the study sample and the original GUiNZ cohort, or the 5,010 children who contributed to the eight-year data collection wave and the original GUiNZ cohort.

[8] Due to multicollinearity between antenatal depression and perceived stress, the authors state that only perceived stress (stronger association with outcome) was used in analysis; however, both variables are listed in the hierarchical linear regression model in study Table 4.

ibuprofen, aspirin, diclofenac, naproxen) and adverse neonatal outcomes" (7). The study examined singleton pregnancies captured in the Aberdeen Maternity and Neonatal Databank (Aberdeen, United Kingdom) between 1985 and 2015. Medical notes were reviewed after delivery for exposure, outcome, and covariate information. Respective use (yes/no) of APAP and other analgesics was captured[9] from medical notes from the patient's first antenatal visit[10]. The study examined twelve different pregnancy or birth outcomes, including, among males only, cryptorchidism (International Classification of Diseases, 10th revision [ICD-10] code Q53) and hypospadias (ICD-10 code Q54).[11] Baseline covariates included year of delivery, maternal age, prior pregnancies, maternal BMI, trimester of first antenatal visit, smoking status, deprivation index, hypertensive disorders, antepartum hemorrhage, labor type, delivery type, use of analgesia during labor, presentation at delivery, and baby sex. Bivariate analysis examined differences in covariates by analgesic use. Crude and adjusted, binary logistic regression was used to examine associations between analgesic use and cryptorchidism and hypospadias.

Among 151,141 pregnancies, 29.1% of pregnancies had exposure to non-prescription analgesics, 18.4% had exposure to APAP alone; 20.0% had exposure to over-the-counter diclofenac alone; and 0.7% of pregnancies had exposure to naproxen, ibuprofen, and aspirin alone. In 1985, 1.3% of pregnancies had APAP exposure, compared to 42.2% in 2015. There were significant differences ($p<0.05$) between analgesic users and non-users for all baseline characteristics. The same was noted for APAP only users and non-users of analgesics, except for baby presentation at delivery and sex of baby. Confounders in adjusted models included clinically relevant covariates associated with the exposure of interest ($p<0.10$). Compared to non-users of analgesics during pregnancy, the use of APAP only was associated with cryptorchidism in crude (OR=1.33; 1.07, 1.66) models but not adjusted models (aOR=0.87; 95% CI: 0.69, 1.09). A similar pattern was noted for hypospadias (crude OR=1.65; 95% CI: 1.31, 2.09; aOR=1.07; 95% CI: 0.84, 1.37). Findings for other exposure categories are presented in Table 1. In adjusted binary/multinomial logistic regression or linear regression models, APAP was significantly associated with preterm delivery, non-live birth pregnancy outcomes, low birth weight, lower standardized birthweight, neonatal intensive care unit admission, and low APGAR scores (one minute and five minute scores).

[9] Zafeiri et al. (2022) article states that women were asked about non-prescription analgesic use at their first antenatal visit.

[10] Timing of visit for those with use of APAP: 79.2% first trimester, 17.1% second trimester, 3.7% third trimester, 0.1% missing; for those with any analgesic use: 83.7% first trimester, 13.2% second trimester, 3.1% first trimester, 0.1% missing; for those with no analgesic use: 65.4% first trimester, 27.4% second trimester, 7.2% third trimester, 0.2% missing

[11] Study also included a composite outcome of congenital anomalies

Table 1. Association between analgesic exposure during pregnancy and selected outcomes[12]

Outcome	At least on analgesic		APAP only		Naproxen/ibuprofen/aspirin only		Diclofenac only (2005-2015)	
	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)	Crude OR (95% CI)	Adjusted OR* (95% CI)
Cryptorchidism	**1.59 (1.35, 1.88)**	0.92 (0.77, 1.11)	**1.33 (1.07, 1.66)**	0.87 (0.69, 1.09)	0.39 (0.05, 2.77)	0.28 (0.04, 1.98)	1.02 (0.76, 1.36)	1.05 (0.78, 1.42)
Hypospadias	**2.35 (1.98, 2.80)**	**1.27 (1.05, 1.54)**	**1.65 (1.31, 2.09)**	1.07 (0.84, 1.37)	**2.70 (1.11, 6.59)**	1.91 (0.78, 4.68)	**1.48 (1.09, 1.99)**	**1.49 (1.09, 2.03)**

*Adjusted for: year of delivery, maternal age at delivery, deprivation index, first gestational booking, gestation at delivery

4 REVIEW DISCUSSION

4.1 SZNAJDER ET AL. (2022)

This prospective, cohort study had a large sample size (~2,400 mother-infant pairs). The FBS study sample, compared to Pennsylvania as a whole, has characteristics suggesting the sample has a higher overall socioeconomic status. This difference may impact study generalizability. Additionally, those who remained in the study to 36-weeks of follow-up differed from those in the original FBS sample, which could introduce selection bias. For example, those who remained in the study were more likely to be White non-Hispanic. White non-Hispanics were more likely to use APAP but less likely to report anxiety/depression or withdrawn behavior problems in their children, which could bias associations for these outcomes toward the null.

The study's exposure assessment had multiple limitations, many that were acknowledged by the study authors. APAP use throughout pregnancy was only assessed at one point in time, during the third trimester. Women were asked to report if they occasionally used any medication at any time since becoming pregnant. This approach may underestimate APAP use as women reported use of APAP from the start of pregnancy through their exposure interview in their third trimester, which, on average, occurred at 35 weeks gestation. Because there were no additional follow-up exposure assessments, this approach would miss capturing any APAP use during pregnancy that occurs between the one-time exposure interview (during the third trimester) and the end of pregnancy. Women may also not remember or report APAP use early in pregnancy. The study was unable to examine dosage, timing (e.g., week or trimester), or frequency of APAP use. APAP use during pregnancy was dichotomized (any use versus no use). Consequently, women classified as having APAP use during pregnancy could include those that used a small dose at one point in time or who had heavy use throughout pregnancy.

[12] Adapted from Zafeiri et al. (2022) Table 2 and Table 3

The study outcome, the CBCL, has been used in five studies (8-12) previously reviewed by DEPI-I.[13] These prior studies varied in whether they examined total scores, internalizing and externalizing summary scores, or syndrome scale scores (as in the Sznajder et al. [2022] study (4)). Studies examined CBCL scores as continuous and/or dichotomous, with those dichotomizing scores typically using T-scores. The current study used the 80th percentile as a cutoff.[14] No associations, in fully adjusted models, were found between APAP use during pregnancy and the CBCL, in three of the prior studies (8, 10, 12). In Brandlistuen et al. (2013), there was a significant association between long-term APAP use during pregnancy (≥28 days) and worse continuous internalizing and externalizing behavior scores (9). No significant associations were found for those with short-term exposure (9). In Trønnes et al. (2020), the only significant association was found between three trimesters of APAP use and internalizing problems (adjusted relative risk=1.36, 95% CI: 1.02, 1.80) (11).

As a major strength, the study considered many covariates as potential confounders. When possible, covariate data were verified with hospital discharge and birth certificate data. There were differences between those who used and did not use APAP during pregnancy. Notably, APAP users were more likely to consume alcohol during pregnancy, take non-prescription drugs, have a diagnosis of anxiety or depression, and report more stress during pregnancy. However, as noted by the authors, no data were collected on possible confounders such as neurobehavioral problems in parents, epigenetics, or APAP use during childhood.

The final, adjusted models adjusted for several confounders, including stress. The authors noted that stress was significantly associated with the outcome in adjusted models and suggested that this association reflects an independent influence of stress on behavioral problems. However, without further context, estimates of association for confounders should not be interpreted in such tables (13). The models did not adjust for key indications, specifically fever and headache/migraine, due to collinearity with APAP use during pregnancy. There was also no stratified analysis by these indications. These indications are not necessarily specific to APAP—they reflect why women took any medication, not just why they took APAP, during pregnancy. Past DEPI-I reviewed studies suggest that use of APAP for fever may be protective for neurobehavioral outcomes.

To aid in the evaluation of the literature, during DEPI-I's last review of the APAP literature, a list of criteria indicative of higher quality studies was developed (see Appendix L). This study does not meet several criteria. This study is not a population-based study, did not conduct any bias or sensitivity analyses, and has limited exposure information. The study did not assess APAP exposure at multiple time points; did not capture APAP exposure that occurred during pregnancy but after the exposure interview; and did not consider dose, frequency, or duration of

[13] Mosholder AD, Taylor LG, Wang C. Neurodevelopmental outcomes following prenatal acetaminophen exposure. October 14, 2016. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 3999031.; Abraham D, Mosholder AD, Leishear White K, Sandhu SK. Functional neurobehavioral outcomes and urogenital outcomes associated with prenatal acetaminophen exposure. July 15, 2022. Silver Spring (MD), U.S. Food and Drug Administration. Available in Lifecycle Signal Tracker (LiST) under NISS ID: 1001355.

[14] Authors based this threshold on that used in a prior study. The scoring manual for the CBCL is copyrighted, so we are unable to comment on how T-scores correspond to percentile thresholds.

use in the analysis. Although the study collected data on multiple indications and covariates, several key indications were omitted from models due to collinearity and covariates in final models were limited to those associated with the exposure and outcome in bivariate analysis.

4.2 THEUNISSEN ET AL. (2022)

The study found an association between APAP use during pregnancy and depressive symptoms at eight years of age. Associations were also noted for maternal stress, maternal smoking, and pre-pregnancy BMI. Depression has not been examined as an outcome in prior studies reviewed by DEPI-I. However, the CBCL, as noted in the Sznajder et al. (2022) methods summary, includes depression (4). In Sznajder et al. (2022), the only significant adjusted association between APAP use during pregnancy and the CBCL was with attention problems and sleep problems (4). Of the five prior studies, reviewed by DEPI-I, that examined CBCL outcomes (8-12), adjusted associations were only noted in two studies, both of which found associations restricted to long-term (≥28 days) (9) or three trimesters of use (11).

Although this study suggests some evidence of an association between APAP use during pregnancy and depressive symptoms, the study has several key limitations. Specifically, APAP exposure during pregnancy was retrospectively assessed at one point in time, typically during the third trimester, and no information was collected on duration, dose, or timing of use. The outcome, although validated and obtained through self-report at a set age, was assessed on a continuous scale. Although the CESD-10 is used to screen for depression, it is unclear if the differences in symptoms noted in the study are clinically meaningful. Although the study adjusted for many socio-demographic and maternal characteristics, there was no adjustment for indication. The study also did not conduct any bias or sensitivity analyses to ascertain robustness of the study findings. Lastly, the study used a predictive modelling approach, which is suited to hypothesis generation, and did not specifically address the etiologic question if APAP use during pregnancy causes childhood depressive symptoms.

4.3 ZAFEIRI ET AL. (2022)

The study found no association, in adjusted models, between APAP use alone and cryptorchidism or hypospadias, compared to non-users of analgesics. A prior DEPI-I review of prenatal APAP and urogenital outcomes[15] found no associations between APAP use during pregnancy and hypospadias (14-16).[16] In Interrante et al. (2017), prenatal non-steroidal anti-inflammatory drugs were associated with a significantly increased odds of hypospadias, compared to APAP users (17). For cryptorchidism, associations were noted in some studies (16, 18, 19) but not all (15, 20).

[15] Mosholder AD, Leishear K, Sandhu SK. Urogenital outcomes with in utero acetaminophen exposure. January 7, 2019. Silver Spring (MD), U.S. Food and Drug Administration. DARRTS Reference ID: 4372262.

[16] An additional study (Reference: Kristensen DM, Hass U, Lesné L. et al. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Hum Reprod 2011 Jan;26:235-244) examined the relationship between analgesics and hypospadias and found no association but it is unclear if the study specifically examined APAP separately.

Although this large study found some associations between analgesics and urogenital outcomes, the study found no associations between APAP use alone and the urogenital outcomes of interest. The study had several limitations. APAP use was assessed only at one point in time, most commonly during the first trimester. Consequently, second and third trimester use was typically not captured. Patients were specifically asked about non-prescription analgesic use at medical visits. Given the study retrospectively assessed APAP use through the review of medical notes, there may be information bias if patients were not explicitly asked about APAP use as a part of routine clinical care. Overall, these limitations may lead to underestimation of APAP use. Information on duration, dose, or timing of use was also not collected. Although the study adjusted for few confounders, it did not account for confounding by indication or use of other medications. The study also did not conduct any bias or sensitivity analyses.

4.4 Overall Discussion

The study by Sznajder et al. (2022) found an association between APAP use during pregnancy and attention problems and sleep problems (4). The latter finding is a new finding in the observational literature. The study also found that stress during pregnancy may be an important confounder. In Theunissen et al. (2022), an association was found between APAP use during pregnancy and depressive symptoms at eight years of age (5). Although this is the first study reviewed by DEPI-I to examine depressive symptoms as an outcome, depression is a component of the CBCL. The study also found that maternal stress was associated with depressive symptoms. The clinical significance of the noted associations in both studies is unclear. Both studies were highly limited by their one-time retrospective assessment of APAP exposure during pregnancy, which was dichotomized, as well as their lack of adjustment or stratification for key indications for APAP use, namely fever and headache/migraine. Five prior studies, previously reviewed by DEPI-I, assessed the relationship between APAP use during pregnancy and child outcomes assessed with the CBCL (8-12). Significant associations were only noted in two prior studies for long durations (≥28 days) or consistent (all three trimesters) use of APAP (9, 11).

Consistent with three prior studies reviewed by DEPI-I (14-16), Zafeiri et al. (2022) found no association between APAP use during pregnancy and hypospadias (7). Zafeiri et al. (2022) also found no association between APAP use during pregnancy and cryptorchidism. Five previous studies reviewed by DEPI-I examined prenatal APAP use and cryptorchidism (15, 16, 18-20), with three finding associations (16, 18, 19). Similar to the Sznajder et. al (2022) (4) and Theunissen et al. (2022) (5) studies of neurobehavioral outcomes, the assessment and operationalization of the exposure and adjustment for confounding, including confounding by indication, was particularly limited.

5 CONCLUSIONS

Overall, findings on the associations between APAP use during pregnancy and neurobehavioral and urogenital outcomes remain mixed. The three studies reviewed here are limited and do not change DEPI-I's conclusions from its most recent review—the limitations and inconsistent

findings of current observational studies of APAP and neurobehavioral and urogenital outcomes are unable to support a determination of causality.

6 APPENDICES

6.1 APPENDIX A. SZNAJDER ET AL. STUDY SUMMARY TABLE

	Study
1.1 Objectives/Aims/Scope	To examine associations between prenatal acetaminophen (APAP) exposure and offspring neurobehavioral problems at the age of 3 years, with focus on the potentially confounding effects of prenatal stress.
1.2.1 Design	
1.2.1.1 Type	Prospective cohort study
1.2.1.2 Data Source	First Baby Study (FBS) – longitudinal cohort study of nulliparous pregnant women
1.2.1.3 Time Period	Delivery between 2009 and 2011
1.2.1.4 Criterion (Selection) Standards	Inclusion Criteria: • Women 18-35 years of age • Speak English or Spanish • Plan to deliver at hospital in Pennsylvania • Completed 36-month postpartum questionnaire • Have some outcome data available with no more than one item skipped on a respective Child Behavior Checklist (CBCL) syndrome scale Exclusion Criteria: • <18 years of age • Prior pregnancy (≥20 weeks gestation) • Planned home delivery or delivery at non-hospital affiliated birthing center • Plan for child to be adopted • Deliver <34 weeks gestation
1.2.1.5 Protected Health Information	Institutional review board approval from Penn State College of Medicine and entities involved in recruitment. Women provided signed, informed consent.
1.2.2 Setting	Pennsylvania
1.2.3 Exposure	Telephone interview in third trimester. APAP exposed if reported taking one or more medications that included APAP.
1.2.4 Outcome(s)	Neurobehavioral problems assessed with the CBCL syndrome scales: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, aggressive behavior. Dichotomized with 80th percentile score as cutoff for problems.
1.2.5 Covariates	• Stress during pregnancy • Depression during pregnancy • Maternal socio-demographic factors • Pre-pregnancy health history

	• Health habits during pregnancy • Co-medications • Reason took medication in pregnancy • Labor induction and mode of delivery • Pregnancy and delivery complications • Newborn characteristics
1.2.6 Sample Size	No power calculations provided. Sample size = 2,422
1.2.7 Statistical Analyses	Two-sided Chi-square tests to compare characteristics by APAP exposure. Unadjusted and adjusted multivariable logistic regression to examine associations between APAP use during pregnancy and CBCL syndrome scale outcomes.
1.2.8 Study Results	1,011 of 2,422 women (41.7%) reported APAP use during pregnancy. After adjustment for confounders, including stress during pregnancy, any APAP use was significantly associated with sleep problems (adjusted odds ratio [aOR]=1.23, 95% confidence interval [CI]=1.01, 1.51) and attention problems (aOR=1.21, 95% CI: 1.01, 1.45) but not emotionally reactive (aOR=0.97, 95% CI: 0.78, 1.20), anxious/depressed (aOR=1.16, 95% CI: 0.94, 1.43), somatic complaints (aOR=1.02, 95% CI: 0.79, 1.32), withdrawn (aOR=1.16, 95% CI: 0.95, 1.42), or aggressive behavior (aOR=1.06, 95% CI: 0.84, 1.34).

6.2 APPENDIX B. MATERNAL AND NEONATAL CHARACTERISTICS OVERALL AND BY ACETAMINOPHEN USE DURING PREGNANCY (N = 2,422).[17]

Characteristic	Overall	Acetaminophen Use During Pregnancy		p-Value
		Yes	No	
		1,011 (41.7%)	1,411 (58.3%)	
	n (%)	n (%)	n (%)	
Reasons women took medications in pregnancy				
Fever	25 (1.1)	25 (2.5)	0.0	< .001
Infection	297 (12.3)	146 (14.4)	151 (10.7)	.006
Muscle pain	381 (15.9)	359 (35.5)	22 (1.6)	< .001
Headache/migraine	693 (28.6)	678 (67.1)	15 (1.1)	< .001
Cold/allergies	465 (19.2)	318 (31.5)	147 (10.4)	< .001
Trouble sleeping	62 (2.6)	49 (4.8)	13 (0.9)	< .001
Thyroid conditions	90 (3.7)	28 (2.8)	62 (4.4)	.037
Anemia	337 (13.9)	129 (12.8)	208 (14.7)	.165
Asthma	98 (4.0)	47 (4.6)	51 (3.6)	.203
Nausea	158 (6.5)	76 (7.5)	82 (5.8)	.094
Maternal age, y				.041
18–24	483 (19.9)	180 (17.8)	303 (21.5)	
25–29	1,041 (43.0)	459 (45.4)	582 (41.2)	
30+	898 (37.1)	372 (36.8)	526 (37.3)	
Maternal education				.289
High school or less	273 (11.3)	104 (10.3)	169 (12.0)	
Some college/technical	619 (25.6)	252 (24.9)	367 (26.0)	
College degree or higher	1,530 (63.2)	655 (64.8)	875 (62.0)	
Married	1,886 (77.9)	805 (79.6)	1,081 (76.6)	.078
White non-Hispanic	2,134 (88.1)	921 (91.1)	1,213 (86.0)	< .001
Private Insurance	2,028 (83.7)	872 (86.3)	1,156 (81.9)	.004
Smoked during pregnancy	190 (7.8)	86 (8.5)	104 (7.4)	.305
Alcohol during pregnancy	231 (9.5)	115 (11.4)	116 (8.2)	.009
Co-medications[a]				
Non-prescription drugs	884 (36.5)	501 (49.6)	383 (27.1)	< .001
Prescription drugs	965 (39.8)	411 (40.7)	554 (39.3)	.491
Diagnosed anxiety or depression	549 (22.7)	253 (25.0)	296 (21.0)	.019
Depressed in pregnancy[b]	123 (5.1)	52 (5.2)	71 (5.0)	.899
Stress in pregnancy[c]				< .001
Low (12–16)	879 (36.3)	324 (32.0)	555 (39.4)	
Medium (17–20)	926 (38.3)	409 (40.5)	517 (36.7)	
High (21+)	614 (25.4)	278 (27.5)	336 (23.9)	
Pre-pregnancy BMI (lk/m^2)				.030
< 25.0	1,371 (56.6)	556 (55.0)	815 (57.8)	
25.0–29.9	551 (22.8)	220 (21.8)	331 (23.5)	
30.0+	499 (20.6)	234 (23.2)	265 (18.8)	
Labor induced	815 (33.6)	367 (36.3)	448 (31.8)	.019
Cesarean Delivery	712 (29.4)	329 (32.5)	383 (27.1)	.004
Indication for Cesarean				
Dystocia	581 (24.0)	264 (26.1)	317 (22.5)	.038
Breech	103 (4.3)	50 (4.9)	53 (3.8)	.133
Other malpresentation	188 (7.8)	85 (8.4)	103 (7.3)	.315
Antepartum bleeding	153 (6.3)	47 (4.6)	106 (7.5)	.004
Fetal distress/heart rate	606 (25.0)	242 (23.9)	364 (25.8)	.297
Fetal intolerance of labor	303 (12.5)	135 (13.4)	168 (11.9)	.289
Hypertension/ preeclampsia	301 (12.4)	134 (13.3)	167 (11.8)	.297
Diabetes	167 (6.9)	65 (6.4)	102 (7.2)	.444
Thyroid disorder	128 (5.3)	51 (5.0)	77 (5.5)	.654
Umbilical cord complications	674 (27.8)	297 (29.4)	377 (26.7)	.150
Premature or prolonged rupture of membranes	192 (7.9)	77 (7.6)	115 (8.2)	.631
Fetal intrauterine growth restriction	75 (3.1)	33 (3.3)	42 (3.0)	.687
Apgar at 5-minutes				.782
1–7	115 (4.7)	45 (4.5)	70 (5.0)	
8	453 (18.7)	186 (18.4)	267 (18.9)	
9–10	1,854 (76.5)	780 (77.2)	1,074 (76.1)	
Gestational age (weeks)				.409
Preterm (34–36)	91 (3.8)	38 (3.8)	53 (3.8)	
Early term (37–38)	465 (19.2)	189 (18.7)	276 (19.6)	
Full term (39–40)	1,471 (60.7)	604 (59.7)	867 (61.4)	
Late term/postterm (41+)	395 (16.3)	180 (17.8)	215 (15.2)	
Birth weight (gms)				.645
<2,500 (underweight)	67 (2.8)	27 (2.7)	40 (2.8)	
2,500–4,000 (normal)	2,082 (85.9)	863 (85.4)	1,219 (86.4)	
>4,000 (macrosomic)	273 (11.4)	121 (12.0)	152 (10.8)	
Male	1,213 (50.1)	512 (50.6)	701 (49.7)	.641
Assisted ventilation	110 (4.5)	54 (5.3)	56 (4.0)	.110
Neonatal ICU (NICU)	122 (5.0)	60 (5.9)	62 (4.4)	.087
Jaundice	540 (22.3)	242 (23.9)	298 (21.1)	.100

[a]Excluding prenatal vitamins and acetaminophen
[b]Score of 13+ on Edinburgh Depression Scale [35]
[c]Psychosocial Hassles Scale [29]
[d]*p*-values were calculated with a X^2 test.

https://doi.org/10.1371/journal.pone.0272593.t001

[17] Study Table 1 and title from Sznajder et al. (2022)

6.3 APPENDIX C. ASSOCIATION OF POTENTIALLY CONFOUNDING VARIABLES WITH EACH OF THE CBCL SYNDROME SCALE OUTCOMES.[18]

Factor	ER %	AD %	SC %	WD %	SP %	AP %	AB %
Overall percent with each outcome	17.1	20.0	16.5	23.7	20.5	30.0	20.8
Fever							
Yes	12.5	16.0	20.0	20.0	16.0	**8.0**	8.0
No	17.2	20.1	16.4	23.8	20.5	**30.3**	21.0
Infection							
Yes	**22.7**	**25.9**	19.1	**29.7**	23.6	33.4	24.7
No	**16.3**	**19.2**	16.1	**22.9**	20.0	29.6	20.3
Muscle pain							
Yes	17.9	19.5	**19.9**	26.2	23.1	31.7	**25.7**
No	17.0	20.1	**15.8**	23.3	20.0	29.4	**19.9**
Headache/migraine							
Yes	18.2	22.3	16.7	25.3	22.3	31.7	23.0
No	16.7	19.1	16.4	23.1	19.7	29.4	20.0
Cold/allergies							
Yes	18.4	22.2	16.4	**27.5**	22.2	31.9	20.6
No	16.8	19.5	16.5	**22.8**	20.1	29.6	20.9
Trouble sleeping							
Yes	14.5	12.9	14.5	22.6	22.6	**43.5**	19.4
No	17.2	20.2	16.5	23.8	20.4	**29.7**	20.9
Thyroid conditions							
Yes	24.7	25.8	18.0	18.9	**30.0**	**43.2**	20.2
No	16.8	19.8	16.4	23.9	**20.1**	**29.5**	20.9
Maternal age, y							
18–24	17.8	**25.0**	**20.7**	**26.5**	21.9	**35.5**	**26.2**
25–29	15.3	**17.9**	**15.4**	**21.0**	19.0	**29.8**	**19.0**
30+	18.9	**19.9**	**15.5**	**25.4**	21.4	**27.4**	**20.0**
Race							
White non-Hispanic	**17.7**	**18.9**	16.3	**23.1**	20.6	29.7	20.7
Minority	**12.7**	**28.3**	17.8	**28.6**	19.2	32.4	22.1
Insurance coverage							
Private	16.9	**18.2**	16.1	**23.0**	20.0	**28.8**	**19.3**
Public	18.2	**29.7**	18.6	**27.7**	23.1	**36.5**	**28.6**
Alcohol during pregnancy							
Yes	**23.6**	**27.9**	**23.8**	**31.2**	**27.3**	**42.4**	**29.4**
No	**16.4**	**19.2**	**15.7**	**22.9**	**19.7**	**28.7**	**19.9**
Non-prescription drugs[a]							
Yes	18.4	21.1	18.0	24.2	22.0	31.7	22.1
No	16.4	19.4	15.6	23.4	19.6	29.1	20.1
Diagnosed anxiety/depression[b]							
Yes	**22.4**	**25.8**	**19.2**	**26.9**	**25.4**	**34.9**	**28.1**
No	**15.6**	**18.4**	**15.7**	**22.8**	**19.0**	**28.6**	**18.7**
Stress during pregnancy[c]							
Low (12–16)	**11.0**	**14.6**	**13.1**	**17.0**	**16.8**	**23.7**	**15.3**
Medium (17–20)	**18.5**	**20.1**	**15.9**	**25.4**	**21.0**	**28.9**	**18.9**
High (21+)	**23.4**	**27.5**	**22.1**	**30.8**	**24.6**	**40.7**	**31.5**
Pre-pregnancy BMI (k/m^2)							
<, 25.0	17.8	21.3	17.1	23.7	19.6	28.3	19.7
25.0–29.9	16.5	17.9	15.6	20.9	20.1	30.8	21.5
30.0+	15.9	19.2	15.7	26.9	23.1	33.8	23.3
Labor induced							
Yes	18.7	20.3	17.7	23.6	21.5	31.7	22.7
No	16.3	19.9	15.9	23.8	19.9	29.2	19.9
Mode of Delivery							
Vaginal	16.7	19.9	**15.3**	23.7	20.4	30.1	20.5
Cesarean	18.1	20.3	**19.4**	23.9	21.7	29.9	21.7
Dystocia							
Yes	14.9	17.5	17.6	23.6	21.7	30.3	19.1
No	17.8	20.8	16.1	23.8	20.1	30.0	21.4
Antepartum bleeding							
Yes	13.2	17.0	15.0	23.5	22.2	28.1	18.3
No	17.4	20.2	16.6	23.7	20.3	30.2	21.0

Values are bolded in order to indicate statistical significance.

ER = emotionally reactive (n = 2,403); AD = anxious/depressed (n = 2,405); SC = somatic complaints (n = 2,409); WD = withdrawn (N = 2,419); SP = sleep problems (n = 2,419); AP = attention problems (n = 2,400); AB = aggressive behavior (n = 2,386).

[a]Excluding vitamins and acetaminophen

[b]Score of 13+ on the Edinburgh Depression Scale Indicates depression [35]

[c]Psychosocial Hassles Scale [34]

https://doi.org/10.1371/journal.pone.0272593.t002

[18] Study Table 2 and title from Sznajder et al. (2022); Percent with each outcome shown

6.4 APPENDIX D. ASSOCIATION BETWEEN ACETAMINOPHEN EXPOSURE DURING PREGNANCY AND NEUROBEHAVIORAL PROBLEMS IN 3-YEAR-OLD CHILDREN.[19]

Neurobehavioral problems	Total n	Percent with outcome	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Emotionally reactive	2,403			
Did not use acetaminophen	1,398	16.7	Reference	Reference
Used acetaminophen	1,005	17.6	1.06 (0.86–1.32)	0.97 (0.78–1.20)
Anxious/depressed	2,405			
Did not use acetaminophen	1,402	19.0	Reference	Reference
Used acetaminophen	1,003	21.5	1.17 (0.96–1.43)	1.16 (0.94–1.43)
Somatic complaints	2,409			
Did not use acetaminophen	1,402	15.7	Reference	Reference
Used acetaminophen	1,007	17.6	1.15 (0.92–1.42)	1.02 (0.79–1.32)
Withdrawn	2,419			
Did not use acetaminophen	1,410	22.1	Reference	Reference
Used acetaminophen	1,009	26.1	**1.25 (1.03–1.51)**	1.16 (.95–1.42)
Sleep problems	2,419			
Did not use acetaminophen	1,410	18.9	Reference	Reference
Used acetaminophen	1,009	22.7	**1.26 (1.04–1.54)**	**1.23 (1.01–1.51)**
Attention problems	2,400			
Did not use acetaminophen	1,398	28.0	Reference	Reference
Used acetaminophen	1,002	32.9	**1.27 (1.06–1.51)**	**1.21 (1.01–1.45)**
Aggressive behavior	2,386			
Did not use acetaminophen	1,389	19.7	Reference	Reference
Used acetaminophen	997	22.5	1.19 (0.97–1.45)	1.06 (0.84–1.34)

Values are bolded in order to indicate statistical significance.

Emotionally reactive adjusted for infection during pregnancy, maternal race, alcohol consumption, diagnosis of anxiety or depression and stress; Anxious/depressed adjusted for infection during pregnancy, maternal age, race, insurance coverage, alcohol consumption, diagnosis of anxiety or depression, and stress; Somatic complaints adjusted for muscle pain, maternal age, alcohol consumption, diagnosis of anxiety or depression, stress, and mode of delivery; Withdrawn adjusted for infection, cold/allergies, maternal age, race, insurance coverage, alcohol consumption, diagnosis of anxiety or depression, and stress; Sleep problems adjusted for thyroid conditions, alcohol consumption, diagnosis of anxiety or depression, and stress; Attention problems adjusted for trouble sleeping, thyroid conditions, maternal age, insurance coverage, alcohol consumption, diagnosis of anxiety or depression and stress; Aggressive behavior adjusted for muscle pain, maternal age, insurance coverage, alcohol consumption, diagnosis of anxiety or depression and stress.

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6.5 APPENDIX E. FULLY ADJUSTED LOGISTIC REGRESSION MODEL, DEPENDENT VARIABLE THE CHILD BEHAVIOR CHECKLIST SYNDROME SCALE "EMOTIONALLY REACTIVE" .[20]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use during pregnancy	0.97 (0.78-1.20)	.76
Maternal infection during pregnancy	1.45 (1.08-1.96)	.015
White, non-Hispanic	1.59 (1.08-2.33)	.018
Alcohol consumed during pregnancy	1.33 (0.95-1.85)	.098
Diagnosed anxiety or depression pre-pregnancy	1.27 (0.99-1.63)	.061
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.76 (1.34-2.31)	< .001
High (21+)	2.30 (1.71-3.08)	< .001

[a]Psychosocial Hassles Scale (34)

OR, odds ratio; CI, confidence interval

[19] Study Table 3 and title from Sznajder et al. (2022)

[20] Study S1 Table and title from Sznajder et al. (2022)

6.6 Appendix F. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Anxious/Depressed".[21]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use during pregnancy	1.16 (0.94-1.43)	.160
Maternal infection during pregnancy	1.37 (1.02-1.82)	.035
White, non-Hispanic	0.67 (0.49-0.92)	.012
Alcohol consumed during pregnancy	1.39 (1.01-1.92)	.041
Diagnosed anxiety or depression pre-pregnancy	1.31 (1.03-1.67)	.028
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.40 (1.09-1.80)	.008
High (21+)	1.83 (1.39-2.41)	< .001
Maternal age, y		
18-24	Ref	
25-29	0.93 (0.68-1.27)	.644
30+	1.13 (0.82-1.57)	.450
Private Insurance	0.66 (0.49-0.91)	.010

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

6.7 Appendix G. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Somatic Complaints".[22]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use during pregnancy	1.02 (0.79-1.32)	.867
White, non-Hispanic	1.02 (0.72-1.44)	.921
Alcohol consumed during pregnancy	1.55 (1.12-2.16)	.009
Diagnosed anxiety or depression pre-pregnancy	1.10 (0.85-1.42)	.480
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.20 (0.92-1.57)	.178
High (21+)	1.65 (1.23-2.20)	< .001
Maternal age, y		
18-24	Ref	
25-29	0.74 (0.55-0.99)	.045
30+	0.75 (0.56-1.02)	.069
Muscle pain during pregnancy	1.21 (0.88-1.67)	.017
Cesarean delivery	1.33 (1.05-1.68)	.017

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

[21] Study S2 Table and title from Sznajder et al. (2022)

[22] Study S3 Table and title from Sznajder et al. (2022)

6.8 Appendix H. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Withdrawn".[23]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use during pregnancy	1.16 (0.95-1.42)	.145
White, non-Hispanic	0.76 (0.56-1.03)	.075
Alcohol consumed during pregnancy	1.31 (0.96-1.77)	.087
Diagnosed anxiety or depression pre-pregnancy	1.05 (0.84-1.33)	.660
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.63 (1.29-2.05)	< .001
High (21+)	2.03 (1.57-2.64)	< .001
Maternal age, y		
18-24	Ref	
25-29	0.86 (0.65-1.16)	.338
30+	1.15 (0.85-1.56)	.366
Maternal infection during pregnancy	1.34 (1.02-1.76)	.037
Cold/allergies during pregnancy	1.18 (0.93-1.51)	.180
Private insurance at childbirth	0.93 (0.68-1.26)	.622

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

6.9 Appendix I. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Sleep Problems".[24]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use during pregnancy	1.23 (1.01-1.51)	.041
Alcohol consumed during pregnancy	1.38 (1.01-1.89)	.044
Diagnosed anxiety or depression pre-pregnancy	1.32 (1.04-1.66)	.021
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.23 (0.97-1.56)	.092
High (21+)	1.45 (1.11-1.89)	.006
Thyroid disorder in pregnancy	1.81 (1.14-2.89)	.012

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

[23] Study S4 Table and title from Sznajder et al. (2022)

[24] Study S5 Table and title from Sznajder et al. (2022)

6.10 Appendix J. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Attention Problems" .[25]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use in pregnancy	1.21 (1.01-1.45)	.038
Alcohol consumed during pregnancy	1.61 (1.21-2.14)	.001
Diagnosed anxiety or depression pre-pregnancy	1.09 (0.88-1.35)	.446
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.23 (0.99-1.52)	.064
High (21+)	1.95 (1.53-2.47)	< .001
Maternal age, y		
18-24	Ref	
25-29	0.87 (0.66-1.13)	.295
30+	0.80 (0.60-1.06)	.114
Thyroid conditions during pregnancy	2.07 (1.33-3.20)	.001
Trouble sleeping during pregnancy	1.63 (0.96-2.76)	.068
Private insurance at childbirth	0.89 (0.67-1.17)	.391

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

6.11 Appendix K. Fully adjusted logistic regression model, dependent variable the Child Behavior Checklist Syndrome Scale "Aggressive Behavior" .[26]

Predictor	OR adjusted (95% CI)	P-value
Acetaminophen use in pregnancy	1.06 (0.84-1.34)	.629
Alcohol consumed during pregnancy	1.41 (1.03-1.92)	.034
Diagnosed anxiety or depression pre-pregnancy	1.37 (1.08-1.73)	.008
Prenatal stress[a]		
Low (12-16)	Ref	
Medium (17-20)	1.21 (0.94-1.56)	.130
High (21+)	2.11 (1.62-2.76)	< .001
Maternal age, y		
18-24	Ref	
25-29	0.83 (0.62-1.12)	.230
30+	0.95 (0.69-1.31)	.759
Muscle pain during pregnancy	1.24 (0.92-1.67)	.160
Private insurance at childbirth	0.78 (0.58-1.06)	.109

[a]Psychosocial Hassles Scale (34)
OR, odds ratio; CI, confidence interval

[25] Study S6 Table and title from Sznajder et al. (2022)

[26] Study S7 Table and title from Sznajder et al. (2022)

6.12 APPENDIX L. STUDY CRITERIA INDICATIVE OF HIGHER QUALITY STUDIES[27]

- Population-based study
- Assessed APAP exposure at multiple time points and captured APAP use throughout pregnancy (ideally allowing more nuanced capture of APAP use [beyond just yes/no])
- Exposure assessed prior to outcome
- Assessed outcomes using a clinical diagnosis, or, if using scale scores, used validated cut-points (ideally considered multiple reporting sources to address subjectivity of responses)
- Ascertained outcomes, when appropriate, at older ages or set ages
- Adjustment for potential confounders, possibly including:
    - Multiple indications (ideally including fever and migraine)
    - Use of other medications during pregnancy
    - Parental education or socio-economic status
    - Use of drugs, alcohol, or tobacco
    - For neurobehavioral studies - genetic factors or, by proxy, relevant familial factors such as parental neurobehavioral conditions (e.g., parental ADHD) or psychiatric conditions
- Conducted analysis to address bias due to unmeasured/residual confounding, selection bias, exposure/outcome misclassification, or missing data

[27] Abraham D, Mosholder AD, Leishear White K, Sandhu SK. Functional neurobehavioral outcomes and urogenital outcomes associated with prenatal acetaminophen exposure. July 15, 2022. Silver Spring (MD), U.S. Food and Drug Administration. Available in Lifecycle Signal Tracker (LiST) under NISS ID: 1001355.

6.13 APPENDIX M. REFERENCES

1. Kwok J, Luedecke E, Hall H, Murray AL, Auyeung B. Analgesic drug use in pregnancy and neurodevelopmental outcomes: an umbrella review. Neurosci Biobehav Rev. 2022 May;136:104607.

2. Patel R, Sushko K, van den Anker J, Samiee-Zafarghandy S. Long-term safety of prenatal and neonatal exposure to paracetamol: A systematic review. Int J Environ Res Public Health. 2022 Feb 14;19(4):2128.

3. Laue HE, Shen Y, Bloomquist TR, Wu H, Brennan KJM, Cassoulet R, et al. In utero exposure to caffeine and acetaminophen, the gut microbiome, and neurodevelopmental outcomes: A prospective birth cohort study. Int J Environ Res Public Health. 2022 Jul 30;19(15):9357.

4. Sznajder KK, Teti DM, Kjerulff KH. Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. PLoS One. 2022 Sep 28;17(9):e0272593.

5. Theunissen G, D'Souza S, Peterson ER, Walker C, Morton SMB, Waldie KE. Prenatal determinants of depressive symptoms in childhood: Evidence from Growing Up in New Zealand. J Affect Disord. 2022 Apr 1;302:41-9.

6. Morton SM, Atatoa Carr PE, Grant CC, Robinson EM, Bandara DK, Bird A, et al. Cohort profile: growing up in New Zealand. Int J Epidemiol. 2013 Feb;42(1):65-75.

7. Zafeiri A, Raja EA, Mitchell RT, Hay DC, Bhattacharya S, Fowler PA. Maternal over-the-counter analgesics use during pregnancy and adverse perinatal outcomes: Cohort study of 151 141 singleton pregnancies. BMJ Open. 2022 May 3;12(5):e048092.

8. Vlenterie R, Wood ME, Brandlistuen RE, Roeleveld N, van Gelder MM, Nordeng H. Neurodevelopmental problems at 18 months among children exposed to paracetamol in utero: A propensity score matched cohort study. Int J Epidemiol. 2016 Dec 1;45(6):1998-2008.

9. Brandlistuen RE, Ystrom E, Nulman I, Koren G, Nordeng H. Prenatal paracetamol exposure and child neurodevelopment: A sibling-controlled cohort study. Int J Epidemiol. 2013 Dec;42(6):1702-13.

10. Tovo-Rodrigues L, Carpena MX, Martins-Silva T, Santos IS, Anselmi L, Barros AJD, et al. Low neurodevelopmental performance and behavioural/emotional problems at 24 and 48 months in Brazilian children exposed to acetaminophen during foetal development. Paediatr Perinat Epidemiol. 2020 May;34(3):278-86.

11. Trønnes JN, Wood M, Lupattelli A, Ystrom E, Nordeng H. Prenatal paracetamol exposure and neurodevelopmental outcomes in preschool-aged children. Paediatr Perinat Epidemiol. 2020 May;34(3):247-56.

12. Parker SE, Collett BR, Werler MM. Maternal acetaminophen use during pregnancy and childhood behavioural problems: Discrepancies between mother- and teacher-reported outcomes. Paediatr Perinat Epidemiol. 2020 May;34(3):299-308.

13. Westreich D, Greenland S. The table 2 fallacy: Presenting and interpreting confounding and modifier coefficients. Am J Epidemiol. 2013 Feb 15;177(4):292-8.

14. Feldkamp ML, Meyer RE, Krikov S, Botto LD. Acetaminophen use in pregnancy and risk of birth defects: Findings from the National Birth Defects Prevention Study. Obstet Gynecol. 2010 Jan;115(1):109-15.

15. Rebordosa C, Kogevinas M, Horváth-Puhó E, Nørgård B, Morales M, Czeizel AE, et al. Acetaminophen use during pregnancy: effects on risk for congenital abnormalities. Am J Obstet Gynecol. 2008 Feb;198(2):178.e1-7.

16. Snijder CA, Kortenkamp A, Steegers EA, Jaddoe VW, Hofman A, Hass U, et al. Intrauterine exposure to mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadia in the offspring: the Generation R Study. Hum Reprod. 2012 Apr;27(4):1191-201.

17. Interrante JD, Ailes EC, Lind JN, Anderka M, Feldkamp ML, Werler MM, et al. Risk comparison for prenatal use of analgesics and selected birth defects, National Birth Defects Prevention Study 1997–2011. Ann Epidemiol. 2017 Oct;27(10):645-53.e2.

18. Kristensen DM, Hass U, Lesné L, Lottrup G, Jacobsen PR, Desdoits-Lethimonier C, et al. Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Hum Reprod 2011 Jan;26:235-44.

19. Jensen MS, Rebordosa C, Thulstrup AM, Toft G, Sørensen HT, Bonde JP, et al. Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. Epidemiology. 2010 Nov;21(6):779-85.

20. Fisher BG, Thankamony A, Hughes IA, Ong KK, Dunger DB, Acerini CL. Prenatal paracetamol exposure is associated with shorter anogenital distance in male infants. Hum Reprod. 2016 Nov;31(11):2642-50.

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